ZooKeys 1189: 33–54 (2024)
DOI: 10.3897/zookeys.1189.115621

Research Article

# A new species of *Amolops* (Amphibia, Anura, Ranidae) from Guizhou Province, China

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## Abstract

The Torrent frogs of the genus *Amolops* are widely distributed in Nepal and northern India eastwards to southern China and southwards to Malaysia. The genus currently contains 84 species. Previous studies indicated underestimated species diversity in the genus. In the context, a new species occurring from the mountains in the northwestern Guizhou Province, China is found and described based on morphological comparisons and molecular phylogenetic analyses, *Amolops dafangensis* **sp. nov.** Phylogenetic analyses based on DNA sequences of the mitochondrial 16S rRNA and COI genes supported the new species as an independent lineage. The uncorrected genetic distances between the 16S rRNA and COI genes in the new species and its closest congener were 0.7% and 2.6%, respectively, which are higher than or at the same level as those among many pairs of congeners. Morphologically, the new species can be distinguished from its congeners by a combination of the following characters: body size moderate (SVL 43.2–46.8 mm in males); head length larger than head width slightly; tympanum distinct, oval; vocal sacs absent; vomerine teeth present; dorsolateral folds weak formed by series of glands; nuptial pads present on the base of finger I; heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level far beyond the tip of the snout when leg stretched forward.

**Key words:** Mitochondrial gene, taxonomy

OPEN ACCESS

Academic editor: Anthony Herrel
Received: 11 November 2023
Accepted: 13 December 2023
Published: 12 January 2024

ZooBank: https://zoobank.org/7351F342-928A-44C1-8EA4-55A706758838

Citation: Li S-Z, Liu J, Ke X-C, Cheng G, Wang B (2024) A new species of *Amolops* (Amphibia, Anura, Ranidae) from Guizhou Province, China. ZooKeys 1189: 33–54. https://doi.org/10.3897/zookeys.1189.115621

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## Introduction

The Torrent frogs of the genus *Amolops* Cope, 1865 are widespread in Asia, from the southern and eastern Himalayas eastward to the southeastern mainland China and southwards to the Peninsular Malaysia (Wu et al. 2020; Zeng et al. 2020; Frost 2023). The frogs live in the fast-flowing water and occupy specialized features that help them cling to rocks and navigate the turbulent currents (Fei et al. 2009; Fei et al. 2012). The genus currently contains 84 species, of which 51 species have been recorded in China (Fei et al. 2012; Amphibia China 2023; Frost 2023). Recently, according to the phylogenetic framework of the genus, the 84 *Amolops* species were divided into ten species groups, namely the *A. monticola*

group, *A. chayuensis* group, *A. hainanensis* group, *A. ricketti* group, *A. spinapectoralis* group, *A. marmoratus* group, *A. larutensis* group, *A. daiyunensis* group, *A. viridimaculatus* group, and the *A. mantzorum* group (Lyu et al. 2019b; Wu et al. 2020; Zeng et al. 2020, 2021; Jiang et al. 2021; Patel et al. 2021; Mahony et al. 2022; Saikia et al. 2022a, 2022b, 2023; Wang et al. 2022; Pham et al. 2023; Qian et al. 2023; Tang et al. 2023; Sheridan et al. 2023). Among them, the *A. mantzorum* group, to which *Amolops dafangensis* sp. nov. belongs, was proposed by Fei et al. (1999) and is mainly distributed along the eastern margin of the Qinghai-Tibet Plateau (Fei et al. 2009; Lu et al. 2014; Zeng et al. 2020) and currently comprises eleven species (Jiang et al. 2021; Qian et al. 2023; Tang et al. 2023): *Amolops ailao* Tang, Sun, Liu, Luo, Yu & Du, 2023, *A. mantzorum* (David, 1872), *A. granulosus* (Liu & Hu, 1961), *A. loloensis* (Liu, 1950), *A. lifanensis* (Liu, 1945), *A. jinjiangensis* Su, Yang & Li, 1986, *A. tuberodepressus* Liu & Yang, 2000, *A. sangzhiensis* Qian, Xiang, Jiang, Yang & Gui, 2023, A. *shuichengnicus* Lyu & Wang, 2019, *A. ottorum* Pham, Sung, Pham, Le, Zieger & Nguyen, 2019, and *A. minutus* Orlov & Ho, 2007. In this species group, *A. ottorum* and *A. minutus* are only known from northwestern Vietnam, and the other species are known from southwestern China (Frost 2023). However, within the group, the phylogenetic relationships between species remain controversial (Lu et al. 2014; Lyu et al. 2019b; Zeng et al. 2020; Wu et al. 2020), and the species diversity of it is also expected to be underestimated (Jiang et al. 2021; Qian et al. 2023; Tang et al. 2023).

Guizhou Province is one of the richest areas for amphibians in China and three *Amolops* species (*A. chaochin*, *A. chunganensis*, and *A. sinensis*) were have been recorded (Amphibia China 2023). During fieldwork in Dafang County, Guizhou Province, some *Amolops* specimens were collected. By our comparisons, these specimens were different from *A. chaochin*, *A. chunganensis*, and *A. sinensis* by the dorsolateral folds being weak, formed by series of glands, and the presence of a circum-marginal groove on the disc of the first finger. Molecular phylogenetic analyses based on mitochondrial DNA and comprehensive morphological comparisons all indicated that the specimens from Dafang County were an undescribed species, herein described as a new species, *Amolops dafangensis* sp. nov.

## Materials and methods

### Sampling

Five specimens of *Amolops dafangensis* sp. nov. including three adult males and two juveniles, were collected from Dafang County, Guizhou Province, China (Fig. 1). All specimens were fixed in 10% buffered formalin for one day, and then transferred to 70% ethanol. Tissue samples were preserved separately in 95% prior to fixation. Specimens collected in this work were all deposited in Maotai Institute (**MT**), Renhuai City, Guizhou Province, China.

### Collection of molecular data

DNA was extracted from tissue using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Two mitochondrial genes, partial 16S ribosomal RNA gene (16S) and cytochrome oxidase subunit I (COI), were amplified.

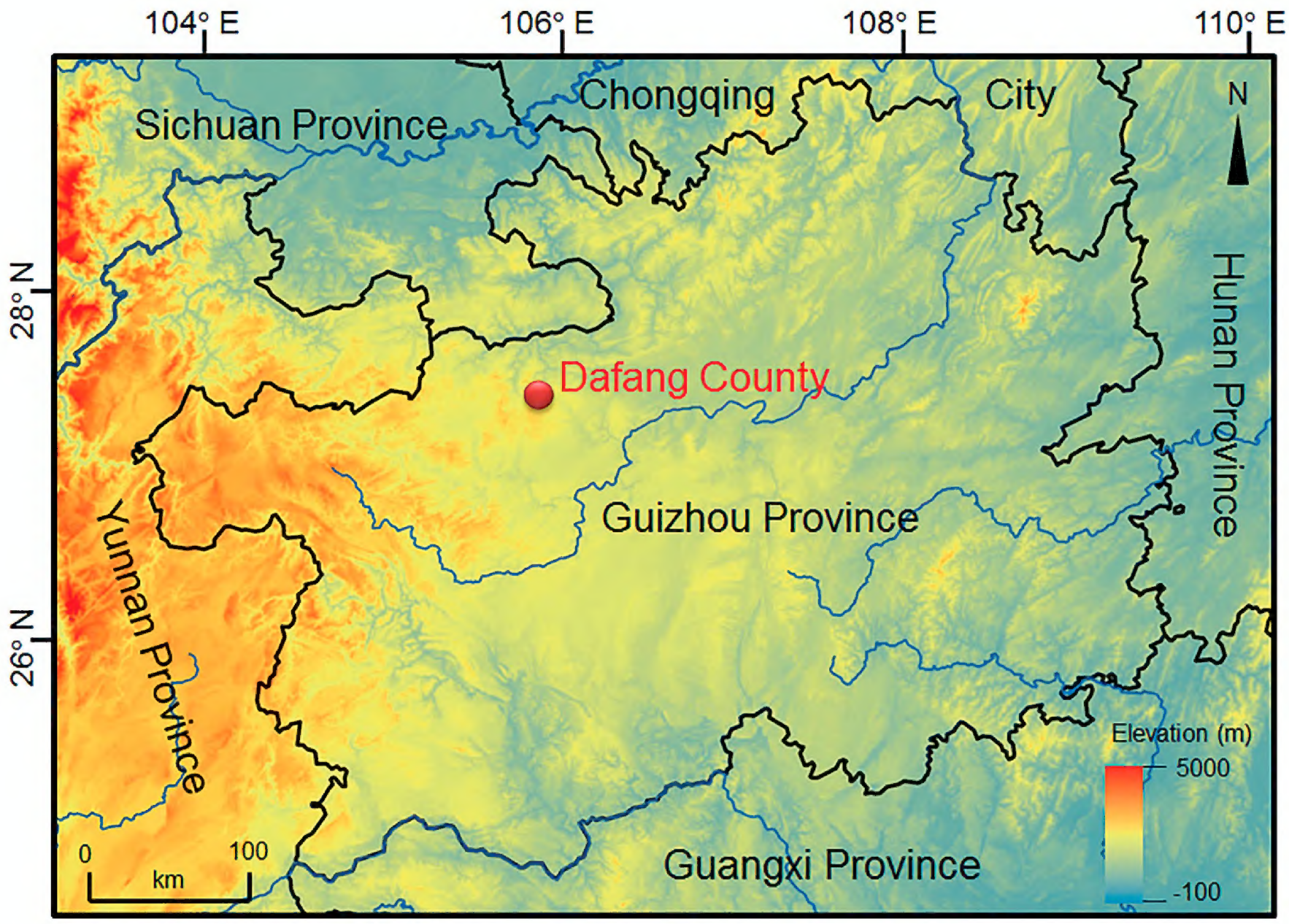


**Figure 1.** Geographical location of the type locality of *Amolops dafangensis* sp. nov. in Dafang County, Guizhou Province, China.

The primers used for 16S were P7 (5'- CGCCTGTTTACCAAAAACAT -3') and P8 (5'-CCGGTCTGAACTCAGATCACGT') following Simon et al. (1994), and that for COI were Chmf4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATCA-3') following Che et al. (2012). PCR amplification reactions were performed in a 30 µl reaction with the following cycling conditions: an initial denaturing step at 95 °C for 4 min; 35 cycles of denaturing at 95 °C for 40 s, annealing at 48 °C/46 °C (16S/COI) for 40 s and extending at 72 °C for 70 s, and a final extending step of 72 °C for 10 min. PCR products were purified with spin columns and then were sequenced with both forward and reverse primers same as PCR. Sequencing was conducted using an ABI Prism 3730 automated DNA sequencer in Chengdu TSING KE Biological Technology Co. Ltd. (Chengdu, China). All sequences were deposited in GenBank (for GenBank Accession numbers refer to Table 1). For phylogenetic analyses, we downloaded corresponding sequences for all related species from GenBank according to previous studies (Qian et al. 2023; Tang et al. 2023; for GenBank accession numbers see Table 1).

## Phylogenetic analyses and genetic distance

Sequences were assembled and aligned using the Clustalw module in BioEdit 7.0.9.0 (Hall 1999) with default settings. The datasets were checked by eye and revised manually if necessary. Based on the 16S + COI concatenated dataset, phylogenetic analyses were conducted using maximum likelihood (ML) and

**Table 1.** Information for samples used in molecular phylogenetic analyses in this study.

| ID | Species | Locality | Voucher number | GenBank accession number | |
|---|---|---|---|---|---|
| | | | | 16S | COI |
| 1 | *Amolops dafangensis* sp. nov. | Dafang, Guizhou, China | MT DF20230601002 | OR936315 | OR924345 |
| 2 | *Amolops dafangensis* sp. nov. | Dafang, Guizhou, China | MT DF20230601001 | OR936314 | OR924344 |
| 3 | *Amolops dafangensis* sp. nov. | Dafang, Guizhou, China | MT DF20230601003 | OR936316 | OR924346 |
| 4 | *Amolops dafangensis* sp. nov. | Dafang, Guizhou, China | MT DF20230601004 | OR936317 | OR924347 |
| 5 | *Amolops dafangensis* sp. nov. | Dafang, Guizhou, China | MT DF20230601005 | OR936318 | OR924348 |
| 6 | *A. mantzorum* | Wolong, Sichuan, China | SCUM 045817HX | MN953706 | MN961408 |
| 7 | *A. mantzorum* | Fengtongzhai, Sichuan, China | SYS a005365 | MK573808 | MK568323 |
| 8 | *A. mantzorum* | Dayi, Sichuan, China | SCUM 045825HX | MN953707 | MN961409 |
| 9 | *A. mantzorum* | Mt. Wawu, Sichuan, China | SYS a005337 | MK604853 | MK605611 |
| 10 | *A. mantzorum* | Kangding, Sichuan, China | KIZ 041127 | MN953764 | MN961465 |
| 11 | *A. mantzorum* | Kangding, Sichuan, China | KIZ 041129 | MN953765 | MN961466 |
| 12 | *A. mantzorum* | Fengtongzhai, Sichuan, China | SYS a005366 | MK604862 | MK605620 |
| 13 | *A. mantzorum* | Kangding, Sichuan, China | SYS a005356 | MK604858 | MK605616 |
| 14 | *A. mantzorum* | Kangding, Sichuan, China | SYS a005357 | MK604859 | MK605617 |
| 15 | *A. mantzorum* | Mt. Wawu, Sichuan, China | SYS a005336 | MK573804 | MK568319 |
| 16 | *A. ailao* | Mt. Ailao, Xinping, Yunnan, China | GXNU YU000001 | MN650752 | MN650738 |
| 17 | *A. ailao* | Mt. Ailao, Xinping, Yunnan, China | GXNU YU000002 | MN650753 | MN650739 |
| 18 | *A. tuberodepressus* | Jingdong, Yunnan, China | SCUM 050433CHX | MN953729 | MN961432 |
| 19 | *A. tuberodepressus* | Mt. Wuliang, Yunnan, China | SYS a003931 | MK573799 | MG991933 |
| 20 | *A. tuberodepressus* | Jingdong, Yunnan, China | SCUM 050430CHX | MN953730 | MN961433 |
| 21 | *A. tuberodepressus* | Mt. Wuliang, Yunnan, China | SYS a003932 | MK573800 | MG991934 |
| 22 | *A. tuberodepressus* | Mt. Ailao, Yunnan, China | SYS a003900 | MK573797 | MK568314 |
| 23 | *A. tuberodepressus* | Mt. Ailao, Yunnan, China | SYS a003901 | MK573798 | MK568315 |
| 24 | *A. granulosus* | Mt. Guangwu, Sichuan, China | SYS a005399 | MK573811 | MK568326 |
| 25 | *A. granulosus* | Mt. Guangwu, Sichuan, China | SYS a005400 | MK573812 | MK568327 |
| 26 | *A. granulosus* | Mt. Wawu, Sichuan, China | SYS a005315 | MK604850 | MK605608 |
| 27 | *A. granulosus* | Mt. Wawu, Sichuan, China | SYS a005316 | MK604851 | MK605609 |
| 28 | *A. granulosus* | China: Dayi, Sichuan | SCUM 045823HX | MN953680 | JN700804 |
| 29 | *A. granulosus* | China: Anxian, Sichuan | SCUM 060911HX | MN953681 | MN961381 |
| 30 | *A. shuichengicus* | Shuicheng, Guizhou, China | SYS a004956 | MK604845 | MK605603 |
| 31 | *A. shuichengicus* | Shuicheng, Guizhou, China | SYS a004957 | MK604846 | MK605604 |
| 32 | *A. jinjiangensis* | Mt. Gaoligong, Yunnan, China | SYS a004571 | MK573801 | MK568316 |
| 33 | *A. jinjiangensis* | Deqing, Yunnan, China | SCUM 050434CHX | MN953700 | MN961402 |
| 34 | *A. jinjiangensis* | Deqing, Yunnan, China | SCUM 050435CHX | EF453741 | MN961403 |
| 35 | *A. jinjiangensis* | Chuxiong, Yunnan, China | KIZ 047905 | MN953701 | MN961404 |
| 36 | *A. loloensis* | Zhaojue, Sichuan, China | SYS a005346 | MK604854 | MK605612 |
| 37 | *A. loloensis* | Zhaojue, Sichuan, China | SYS a005347 | MK604855 | MK605613 |
| 38 | *A. loloensis* | Xichang, Sichuan, China | SCUM 045806HX | MN953704 | MN961407 |
| 39 | *A. loloensis* | Xichang, Sichuan, China | SCUM 045807HX | EF453743 | MN961456 |
| 40 | *A. sangzhiensis* | Mt. Doupeng, Sangzhi, Hunan, China | CSUFT 901 | OQ079538 | OQ078903 |
| 41 | *A. sangzhiensis* | Mt. Doupeng, Sangzhi, Hunan, China | CSUFT 907 | OQ079540 | OQ078905 |
| 42 | *A. sangzhiensis* | Mt. Doupeng, Sangzhi, Hunan, China | CSUFT 912 | OQ079541 | OQ078906 |

| ID | Species | Locality | Voucher number | GenBank accession number | |
|---|---|---|---|---|---|
| | | | | 16S | COI |
| 43 | *A. sangzhiensis* | Mt. Doupeng, Sangzhi, Hunan, China | CSUFT 916 | OQ079542 | OQ078907 |
| 44 | *A. sangzhiensis* | Mt. Doupeng, Sangzhi, Hunan, China | CSUFT 927 | OQ079543 | OQ078908 |
| 45 | *A. sangzhiensis* | Mt. Doupeng, Sangzhi, Hunan, China | CSUFT 930 | OQ079544 | OQ078909 |
| 46 | *A. sangzhiensis* | Mt. Doupeng, Sangzhi, Hunan, China | CSUFT 933 | OQ079545 | OQ078910 |
| 47 | *A. lifanensis* | Lixian, Sichuan, China | SYS a005374 | MK573809 | MK568324 |
| 48 | *A. lifanensis* | Lixian, Sichuan, China | SYS a005375 | MK573810 | MK568325 |
| 49 | *A. lifanensis* | Maoxian, Sichuan, China | SCUM 045801HX | MN953702 | MN961405 |
| 50 | *A. lifanensis* | Maoxian, Sichuan, China | SCUM 045803HX | MN953703 | MN961406 |
| 51 | *A. chunganensis* | Mt. Jinggang, Jiangxi, China | SYS a004212 | MK263263 | MG991914 |
| 52 | *A. ricketti* | Mt. Wuyi, Fujian, China | SYS a004141 | MK263259 | MG991927 |

Bayesian Inference (BI) methods, implemented in PhyML 3.0 (Guindon et al. 2010) and MrBayes 3.12 (Ronquist and Huelsenbeck 2003), respectively. The best-fit model was obtained by the Bayesian inference criteria (BIC) computed with PartitionFinder 2 (Lanfear et al. 2012). In this analysis, 16S gene and each codon position of COI gene were defined, and Bayesian Inference Criteria was used. As a result, the analysis suggested that the best partition scheme is16S gene/each codon position of COI gene, and selected GTR + G + I model as the best model for each partition. For ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to estimate nodal supports of inferred relationships on phylogenetic trees. For Bayesian analyses, four Markov chains were run for 50 million generations with sampling every 1000 generations. The first 25% of the trees were discarded, representing the burn-in phase of the analyses, and the remaining trees were used to calculate the Bayesian posterior probabilities. Genetic distance between species of *A. mantzorum* group were estimated on 16S and COI genes, respectively, based on uncorrected *p*-distance model using MEGA 6.06 (Tamura et al. 2013).

## Morphological comparisons

Morphological measurements were made with dial calipers to nearest 0.1 mm by S-ZL following Fei et al. (2009). In total, twenty morphological characteristics were measured for the adult specimens:

**ED** eye diameter (distance from the anterior corner to the posterior corner of the eye);
**FL** foot length (distance from tarsus to the tip of fourth toe);
**HDL** head length (distance from the tip of the snout to the articulation of jaw);
**HDW** maximum head width (greatest width between the left and right articulations of jaw);
**HLL** hindlimb length (maximum length from the vent to the distal tip of the Toe IV);
**IND** internasal distance (minimum distance between the inner margins of the external nares);
**IOD** interorbital distance (minimum distance between the inner edges of the upper eyelids);

**LAL** length of lower arm and hand (distance from the elbow to the distal end of the Finger IV);
**ML** manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
**NED** nasal to eye distance (distance between the nasal and the anterior corner of the eye);
**NSD** nasal to snout distance (distance between the nasal the posterior edge of the vent);
**LW** lower arm width (maximum width of the lower arm);
**SVL** snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
**SL** snout length (distance from the tip of the snout to the anterior corner of the eye);
**TFL** length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV);
**THL** thigh length (distance from vent to knee);
**TL** tibia length (distance from knee to tarsus);
**TW** maximal tibia width;
**TYD** maximal tympanum diameter;
**UEW** upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

We also compared the morphological characters of the new taxon with other species of *Amolops*. Comparative data were obtained from the literature for all species of *Amolops* (Table 2).

## Results

### Phylogenetic analyses

The ML and BI phylogenetic trees were constructed based on concatenated DNA sequences of the mitochondrial 16S (425 bp) and COI (606 bp) genes. ML and BI analyses resulted in essentially identical topologies though some basal relationships between clades were not resolved (Fig. 2). The new taxon was indicated as an independent clade. Furthermore, the smallest uncorrected *p*-distance between *Amolops dafangensis* sp. nov. and its most closely-related congeners is 0.7% (vs *A. sangzhiensis*) on 16S gene (Suppl. material 1), and 2.6% (vs *A. loloensis*) on COI gene (Suppl. material 2), which was higher or at the same level with those among many pairs of congeners, for example, 0.3% between *A. sangzhiensis* and *A. jinjiangensis* on the 16S gene, and 3.2% between *A. jinjiangensis* and *A. loloensis* on the COI gene.

### Morphological comparisons

Morphological measurements are given in Table 3. The new taxon could be identified from its congeners by a series of differences in morphological characters.

**Table 2.** References for morphological characters for congeners of the genus *Amolops*.

| Species | Literature |
|---|---|
| *A. adicola* Patel, Garg, Das, Stuart & Biju, 2021 | Patel et al. 2021 |
| *A. afghanus* (Günther, 1858) | Günther 1858 |
| *A. ailao* Tang, Sun, Liu, Luo, Yu & Du, 2023 | Tang et al. 2023 |
| *A. akhaorum* Stuart, Bain, Phimmachak & Spence, 2010 | Stuart et al. 2010 |
| *A. albispinus* Sung, Wang & Wang, 2016 | Sung et al. 2016 |
| *A. aniqiaoensis* Dong, Rao & Lü, 2005 | Zhao et al. 2005 |
| *A. archotaphus* (Inger & Chan-ard, 1997) | Inger and Chan-ard 1997 |
| *A. attiguus* Sheridan, Phimmachak, Sivongxay & Stuart, 2023 | Sheridan et al. 2023 |
| *A. assamensis* Sengupta, Hussain, Choudhury, Gogoi, Ahmed & Choudhury, 2008 | Sengupta et al. 2008 |
| *A. australis* Chan, Abraham, Grismer & Grismer, 2018 | Chan et al. 2018 |
| *A. beibengensis* Jiang, Li, Zou, Yan & Che, 2020 | Che et al. 2020 |
| *A. bellulus* Liu, Yang, Ferraris & Matsui, 2000 | Liu et al. 2000 |
| *A. binchachaensis* Rao, Hui, Ma & Zhu, 2022"2020" | Zhu and Rao 2022 |
| *A. chakrataensis* Ray, 1992 | Ray 1992 |
| *A. chanakya* Saikia, Laskar, Dinesh, Shabnam & Sinha, 2022 | Saikia et al. 2022a |
| *A. chaochin* Jiang, Ren, Lyu & Li, 2021 | Jiang et al. 2021 |
| *A. chayuensis* Sun, Luo, Sun & Zhang, 2013 | Sun et al. 2013 |
| *A. chunganensis* (Pope, 1929) | Pope 1929 |
| *A. compotrix* (Bain, Stuart & Orlov, 2006) | Bain et al. 2006 |
| *A. cremnobatus* Inger and Kottelat, 1998 | Inger and Kottelat 1998 |
| *A. cucae* (Bain, Stuart & Orlov, 2006) | Bain et al. 2006 |
| *A. daiyunensis* (Liu & Hu, 1975) | Liu and Hu 1975 |
| *A. daorum* (Bain, Lathrop, Murphy, Orlov & Ho, 2003) | Bain et al. 2003 |
| *A. deng* Jiang, Wang & Che, 2020 | Che et al. 2020 |
| *A. formosus* (Günther, 1876) | Günther 1876 "1875" |
| *A. gerbillus* (Annandale, 1912) | Annandale 1912 |
| *A. gerutu* Chan, Abraham, Grismer & Grismer, 2018 | Chan et al. 2018 |
| *A. granulosus* (Liu & Hu, 1961) | Liu and Hu 1961 |
| *A. hainanensis* (Boulenger, 1900) | Boulenger 1900 "1899" |
| *A. himalayanus* (Boulenger, 1888) | Boulenger 1888 |
| *A. hongkongensis* (Pope & Romer, 1951) | Pope and Romer 1951 |
| *A. indoburmanensis* Dever, Fuiten, Konu & Wilkinson, 2012 | Dever et al. 2012 |
| *A. iriodes* (Bain & Nguyen, 2004) | Bain and Nguyen 2004 |
| *A. jaunsari* Ray, 1992 | Ray 1992 |
| *A. jinjiangensis* Su, Yang & Li, 1986 | Su et al. 1986 |
| *A. kaulbacki* (Smith, 1940) | Smith 1940 |
| *A. kohimaensis* Biju, Mahony & Kamei, 2010 | Biju et al. 2010 |
| *A. kottelati* Sheridan, Phimmachak, Sivongxay & Stuart, 2023 | Sheridan et al. 2023 |
| *A. larutensis* (Boulenger, 1899) | Boulenger 1899a |
| *A. latopalmatus* (Boulenger, 1882) | Boulenger 1882 |
| *A. lifanensis* (Liu, 1945) | Liu 1945 |
| *A. loloensis* (Liu, 1950) | Liu 1950 |
| *A. longimanus* (Andersson, 1939) | Andersson 1939 "1938" |
| *A. mahabharatensis* Khatiwada, Shu, Wang, Zhao, Xie & Jiang, 2020 | Khatiwada et al. 2020 |
| *A. mantzorum* (David, 1872) | David 1872 "1871" |
| *A. marmoratus* (Blyth, 1855) | Blyth 1855 |
| *A. medogensis* Li & Rao, 2005 | Zhao et al. 2005 |

| Species | Literature |
|---|---|
| *A. mengdingensis* Yu, Wu & Yang, 2019 | Yu et al. 2019 |
| *A. mengyangensis* Wu & Tian, 1995 | Wu and Tian 1995 |
| *A. minutus* Orlov & Ho, 2007 | Orlov and Ho 2007 |
| *A. monticola* (Anderson, 1871) | Anderson 1871 |
| *A. nepalicus* Yang, 1991 | Yang 1991 |
| *A. nidorbellus* Biju, Mahony & Kamei, 2010 | Biju et al. 2010 |
| *A. nyingchiensis* Jiang, Wang, Xie, Jiang & Che, 2016 | Jiang et al. 2016 |
| *A. ottorum* Pham, Sung, Pham, Le, Ziegler & Nguyen, 2019 | Pham et al. 2019 |
| *A. pallasitatus* Qi, Zhou, Lyu, Lu & Li, 2019 | Qi et al. 2019 |
| *A. panhai* Matsui & Nabhitabhata, 2006 | Matsui and Nabhitabhata 2006 |
| *A. putaoensis* Gan, Qin, Lwin, Li, Quan, Liu & Yu, 2020 | Gan et al. 2020b |
| *A. ricketti* (Boulenger, 1899) | Boulenger 1899b |
| *A. sangzhiensis* Qian, Xiang, Jiang, Yang & Gui, 2023 | Qian et al. 2023 |
| *A. senchalensis* Chanda, 1987 | Chanda 1987 |
| *A. sengae* Sheridan, Phimmachak, Sivongxay & Stuart, 2023 | Sheridan et al. 2023 |
| *A. shihaitaoi* Wang, Li, Du, Hou & Yu, 2022 | Wang et al. 2022 |
| *A. shuichengicus* Lyu & Wang, 2019 | Lyu et al. 2019a |
| *A. siju* Saikia, Sinha, Shabnam & Dinesh, 2023 | Saikia et al. 2023 |
| *A. sinensis* Lyu, Wang & Wang, 2019 | Lyu et al. 2019b |
| *A. spinapectoralis* Inger, Orlov & Darevsky, 1999 | Inger et al. 1999 |
| *A. tanfuilianae* Sheridan, Phimmachak, Sivongxay & Stuart, 2023 | Sheridan et al. 2023 |
| *A. tawang* Saikia, Laskar, Dinesh, Shabnam & Sinha, 2022 | Saikia et al. 2022a |
| *A. teochew* Zeng, Wang, Lyu & Wang, 2021 | Zeng et al. 2021 |
| *A. terraorchis* Saikia, Sinha, Laskar, Shabnam & Dinesh, 2022 | Saikia et al. 2022b |
| *A. tonkinensis* (Ahl, 1927 "1926") | Ahl 1927 "1926" |
| *A. torrentis* (Smith, 1923) | Smith 1923 |
| *A. truongi* Pham, Pham, Ngo, Sung, Ziegler & Le, 2023 | Pham et al. 2023 |
| *A. tuanjieensis* Gan, Yu & Wu, 2020 | Gan et al. 2020a |
| *A. tuberodepressus* Liu & Yang, 2000 | Liu and Yang 2000 |
| *A. viridimaculatus* (Jiang, 1983) | Jiang 1983 |
| *A. vitreus* (Bain, Stuart & Orlov, 2006) | Bain et al. 2006 |
| *A. wangyali* Mahony, Nidup, Streicher, Teeling & Kamei, 2022 | Mahony et al. 2022 |
| *A. wangyufani* Jiang, 2020 | Che et al. 2020 |
| *A. wenshanensis* Yuan, Jin, Li, Stuart & Wu, 2018 | Yuan et al. 2018 |
| *A. wuyiensis* (Liu & Hu, 1975) | Liu and Hu 1975 |
| *A. yatseni* Lyu, Wang & Wang, 2019 | Lyu et al. 2019 |
| *A. yunkaiensis* Lyu, Wang, Liu, Zeng & Wang, 2018 | Lyu et al. 2018 |

## Taxonomic account

***Amolops dafangensis* sp. nov.**

https://zoobank.org/22D19386-8779-4FBC-8BF9-71FB7070403B

Figs 3–5

**Material examined. *Holotype*.** MT DF20230601002, adult male, collected by Shize Li on 1 June 2023 in Dafang County (27.40078312°N, 105.92804027°E; elevation 1300 m a.s.l.), Guizhou Province, China. ***Paratypes*.** One male MT DF20230601003 collected by Jing Liu on 1 June 2023, one male MT DF20230601001 and two juveniles MT DF20230601004 and MT DF20230601005 were collected by Xiaocong Ke on 1 June 2023 from the same place as holotype.

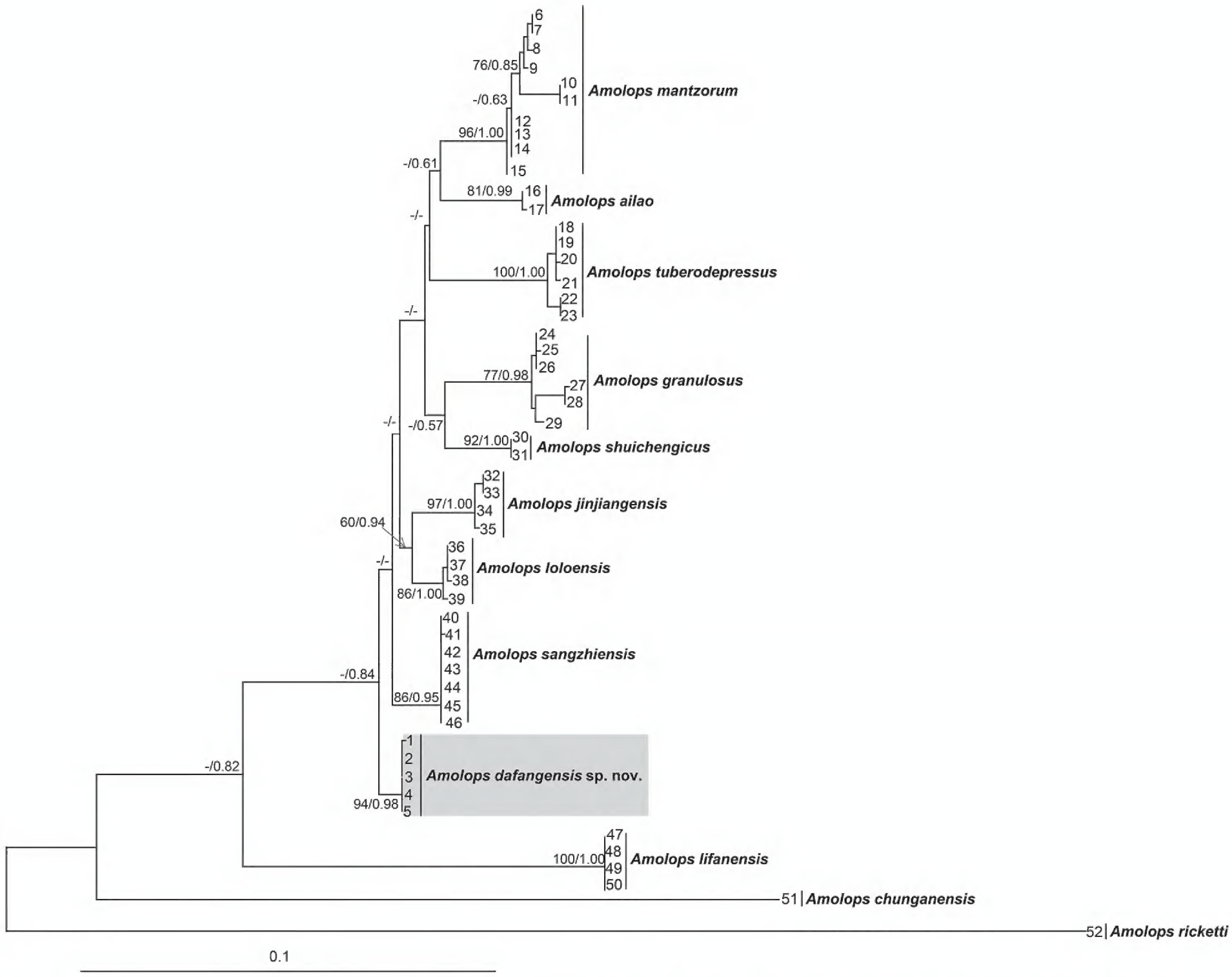


**Figure 2.** Maximum likelihood (ML) tree of *Amolops mantzorum* group based on the 16S and CO1 genes. ML bootstrap supports (BS) /Bayesian posterior probability (BPP) were denoted beside each node, and "-" denotes BS < 50% or BPP < 0.60. Samples 1–52 refer to Table 1.

**Diagnosis.** *Amolops dafangensis* sp. nov. resembles members of the *A. mantzorum* group in the absence of true dorsolateral folds and the presence of a circum-marginal groove on the disc of the first finger. The tarsal fold and tarsal glands are absent, and a nuptial pad is present on the first finger in males (Jiang et al. 2021).

*Amolops dafangensis* sp. nov. can be distinguished from other congeners by the following characters: (1) body size moderate (SVL 43.2 – 46.8 mm in males); (2) head length larger than head width slightly; (3) tympanum distinct, oval; (4) vocal sacs absent; (5) vomerine teeth present; (6) dorsolateral folds weak formed by series of glands; (7) nuptial pads present on base of finger I; (8) heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level far beyond the tip of the snout when leg stretched forward.

**Description of holotype. Adult male** (Figs 3, 4), body size moderate, SVL 44.7 mm. head length larger than head width slightly (HDL: HDW = 1.02); snout short, rounded in dorsal view, projecting beyond lower jaw; eye large and convex, eye diameter 0.74× of snout length; nostril rounded, between to tip of snout and eyes; internasal distance larger than interorbital distance; tympanum circular,

**Table 3.** Measurements of the adult specimens of *Amolops dafangensis* sp. nov. Units are given in mm. See abbreviations for the morphological characters in Materials and methods section.

| Voucher | MT DF20230601001 | MT DF20230601002 | MT DF20230601003 | Range | Mean ± SD |
|---|---|---|---|---|---|
| Sex | male | male | male | | |
| SVL | 43.2 | 44.7 | 46.8 | 43.2–46.8 | 44.9 ± 1.8 |
| HDL | 14.5 | 15.0 | 15.6 | 14.5–15.6 | 14.9 ± 0.6 |
| HDW | 14.3 | 14.7 | 15.1 | 14.3–15.1 | 14.8 ± 0.4 |
| SL | 6.1 | 6.1 | 6.6 | 6.1–6.6 | 6.3 ± 0.3 |
| ED | 3.9 | 4.5 | 4.3 | 3.9–4.5 | 4.3 ± 0.3 |
| UEW | 3.5 | 3.9 | 3.8 | 3.5–3.9 | 3.7 ± 0.2 |
| IOD | 4.4 | 4.1 | 4.7 | 4.1–4.7 | 4.4 ± 0.3 |
| IND | 5.2 | 5.4 | 5.7 | 5.2–5.7 | 5.4 ± 0.3 |
| NED | 2.7 | 2.4 | 3.0 | 2.4–3.0 | 2.7 ± 0.3 |
| NSD | 3.2 | 2.4 | 3.0 | 2.4–3.2 | 2.9 ± 0.4 |
| TYD | 1.9 | 2.4 | 1.7 | 1.7–2.4 | 2.0 ± 0.4 |
| LAL | 22.5 | 24.0 | 23.5 | 22.5–24.0 | 23.3 ± 0.8 |
| LW | 3.2 | 3.8 | 3.8 | 3.2–3.8 | 3.6 ± 0.3 |
| ML | 13.8 | 14.4 | 14.6 | 13.8–14.6 | 14.3 ± 0.4 |
| HLL | 80.4 | 83.4 | 87.3 | 80.4–87.3 | 83.7 ± 3.4 |
| THL | 22.3 | 24.0 | 24.9 | 22.3–24.9 | 23.7 ± 1.3 |
| TL | 25.8 | 26.2 | 27.9 | 25.8–27.9 | 26.6 ± 1.1 |
| TW | 5.0 | 5.4 | 5.8 | 5.0–5.8 | 5.4 ± 0.4 |
| TFL | 36.3 | 38.1 | 39.5 | 36.3–39.5 | 38.0 ± 1.6 |
| FL | 22.3 | 22.8 | 24.6 | 22.3–24.6 | 23.2 ± 1.2 |

distinct, 0.56× of eye diameter; loreal region slightly concave; nares oval; pineal ocellus visible; supratympanic fold extends from back of eye to above shoulder; vomerine teeth present; tongue deeply notched posteriorly; vocal sac absent.

Forelimbs robust (LW/SVL=0.08); lower arm and hand beyond one-second of body length (LAL/SVL=0.51); fingers slender, relative finger lengths I < II < IV < III; finger tips on II–IV dilated to wide cordiform disks with circum-marginal grooves, tip of first finger with small disk but without circum-marginal groove; all fingers without webbing and lateral fringes; subarticular tubercle prominent; supernumerary tubercle indistinct; inner metacarpal tubercle oval, elongate; outer metacarpal tubercles small round; velvety nuptial pad on finger I.

Hindlimbs long, nearly 2× SVL (HLL/SVL = 1.87); tibiotarsal articulation reaching the level far beyond the tip of the snout when leg stretched forward; tibias longer than thigh length, heels overlapped; toes slender, relative lengths I < II < III < V < IV; toes entirely webbed; tips of toes expanded into disc with circum-marginal grooves; outer metatarsal tubercle absent; inner metatarsal tubercle small but well developed.

Skin on dorsum and dorsal surfaces of limbs smooth; dorsolateral folds weak, formed by series of glands been an incomplete line, extending from above shoulder to vent; weak dorsolateral glandular lines; ventral surface of bell and limbs smooth except a few small tubercles on posterior surface of thigh and around vent.

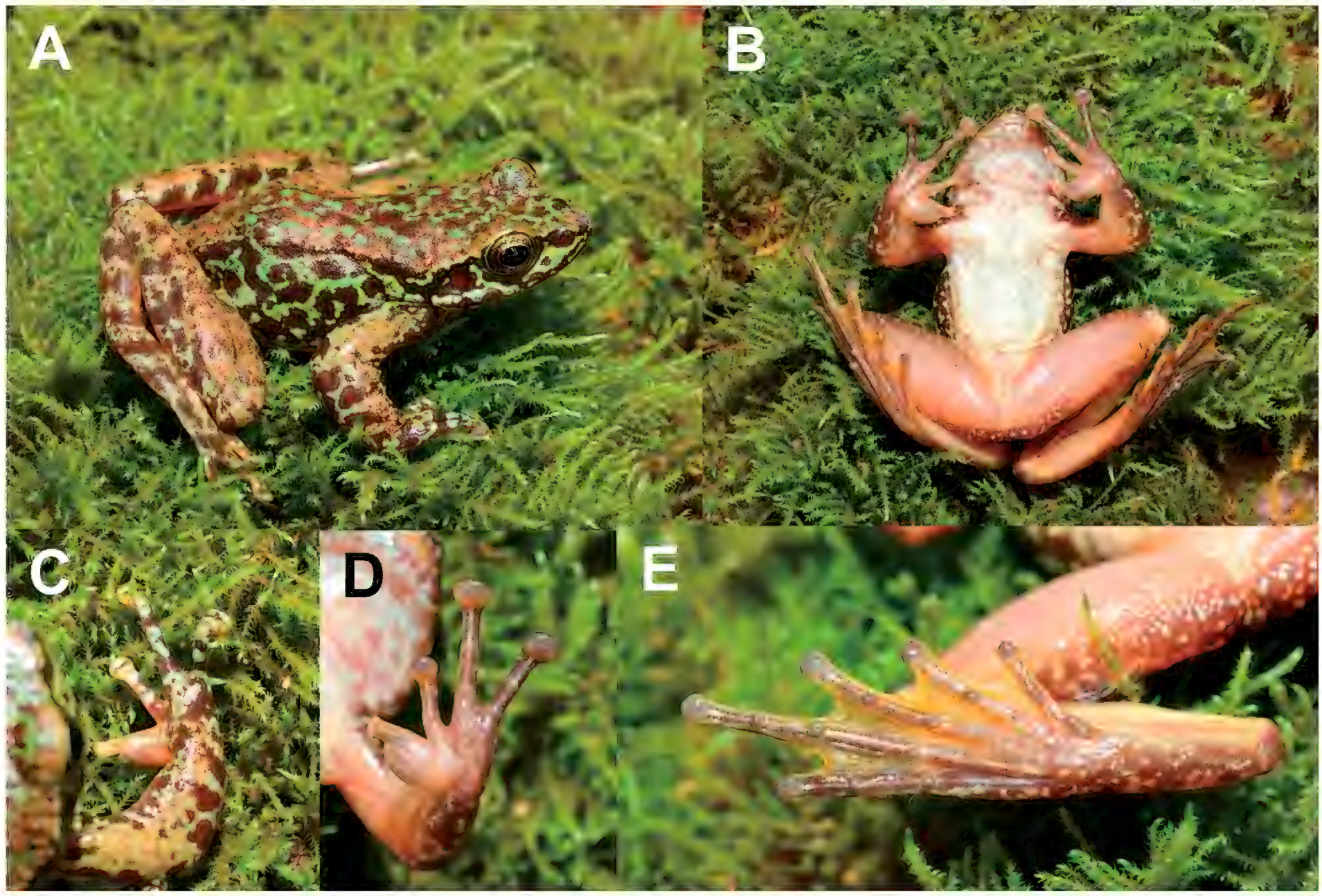


**Figure 3.** Photographs of the holotype MT DF20230601002 of *Amolops dafangensis* sp. nov. in life **A** dorsal view **B** ventral view **C** dorsal view of hand **D** ventral view of hand **E** ventral view of foot.

**Coloration in life.** In life, iris pale brown with dark wash; top of head and dorsum golden brown with large rounded black brown and green spots; sides of head with a pale green stripe extending from loreal region to region behind and below eye along upper lip; a black brown band from the tip of the snout through the nostril to an anterior border of the eye, continuing behind the eye to the shoulder; temporal region black brown with green blotches; the flank green with some back brown spots; limbs dorsally golden brown with black brown bands; chest and venter white, throat white with pale brown; ventral surface of anterior forelimbs brown with green spots; finger I and II fresh-colored, finger III and IV brown; ventral surface of hindlimbs fresh-colored (Fig. 3).

**Color in preservative.** Dorsal surface fade to pale brown with beige brown and black spots on head, flank and on limbs; ventral surface fade to creamy white, marbled with brown on throat and chest (Fig. 4).

**Variation.** Measurements of all specimens are listed in Table 3. All specimens were very similar in morphology, but in MT DF20230601001 the dorsum was golden brown with few green spots (Fig. 5A); in MT DF20230601003 the dorsum and dorsal surfaces of limbs were green with brown spots (Fig. 5B); in the juvenile specimen MT DF20230601004 the flank was mainly green with black spots and the ventral surface of the throat and chest were white with pale brown spots (Fig. 5C, D).

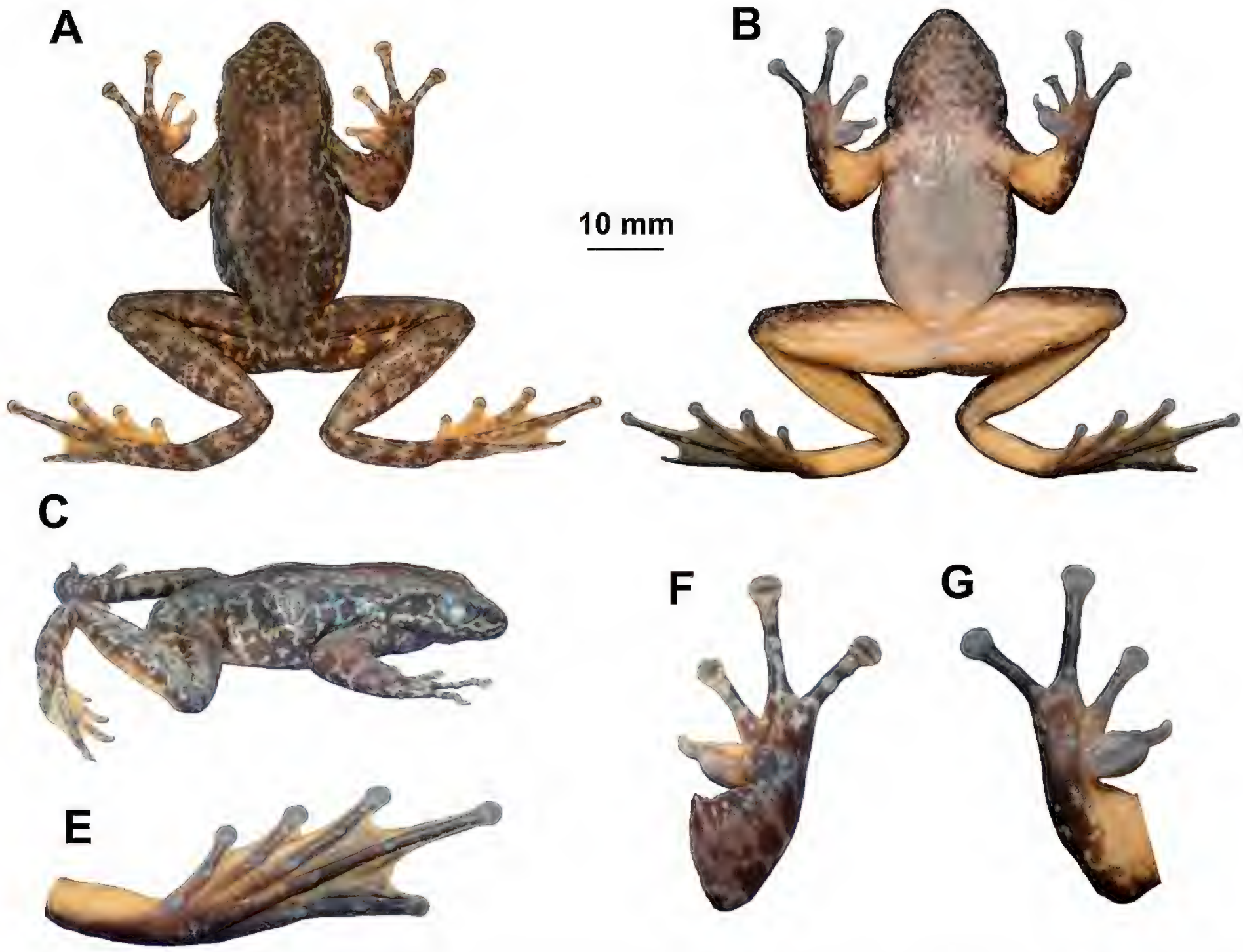


**Figure 4.** The holotype specimen MT DF20230601002 of *Amolops dafangensis* sp. nov. **A** dorsal view **B** ventral view **C** lateral view **D** dorsal view of hand **E** ventral view of hand **F** ventral view of foot.

**Secondary sexual characteristics.** Adult males lack vocal sacs. In breeding, pale yellow glandular nuptial pads are present on finger I in males.

**Morphological comparisons.** The molecular phylogenetic results placed the new species as an independent clade into *A. marmoratus* group. Within the *A. mantzorum* group, the new species can be distinguished from *A. ailao* by having a larger body size (adult males SVL 43.2–46.8 mm vs 33.0–35.1 mm); by vomerine teeth present (vs absent), and by tibiotarsal articulation reaching the level far beyond the tip of the snout when leg stretched forward (vs reaching beyond anterior corner of eye); differs from *A. granulosus* by having a smooth dorsum skin (vs rough with spinules in males) and the absence of vocal sacs in males (vs present); differs from *A. lifanensis* by having a smaller body size (adult males SVL 43.2–46.8 mm vs 52.0–56.0) and having distinct tympanum (vs indistinct); differs from *A. mantzorum* by having a smaller body size (adult males SVL 43.2 – 46.8 mm vs 49.0–57.0 mm), head length about equal to or larger than head width (vs head length smaller than head width); differs from *A. minutus* by having a larger body size (adult males SVL 43.2–46.8 mm vs 29.70–36.42 mm), and the absence of vocal sacs and gular pouches in males (vs well developed); differs from *A. ottorum* by the presence of vomerine teeth (vs absent); differs from *A. shuichengicus* by having a larger body size in males (adult males SVL 43.2–46.8 mm vs 34.6–39.6 mm), and having

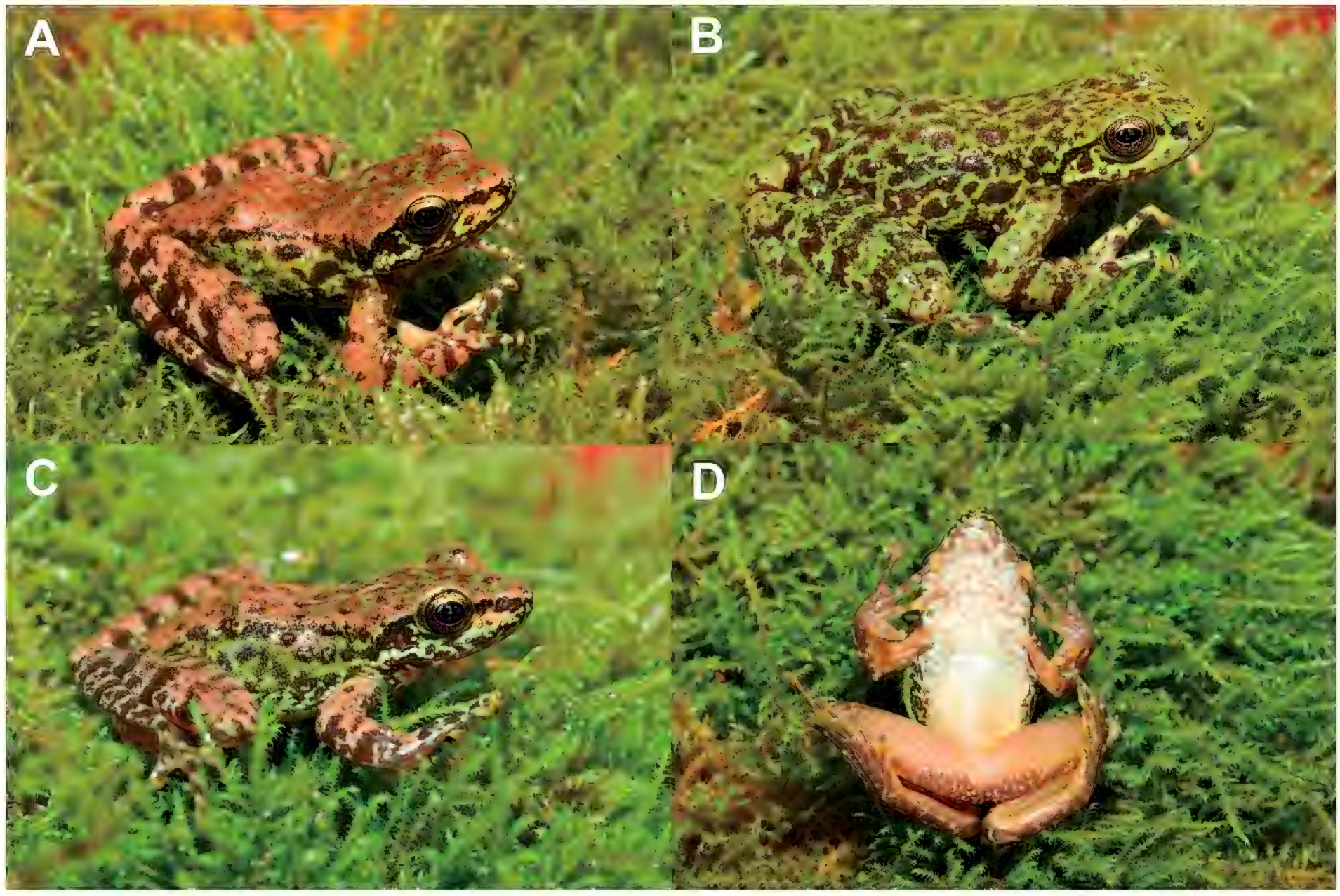


**Figure 5.** Color variation in *Amolops dafangensis* sp. nov. **A** dorsolateral view of the male specimen MT DF20230601001 **B** dorsolateral view of the male specimen MT DF20230601003 **C** dorsolateral view of the juvenile specimen MT DF20230601004 **D** ventral view of the male specimen juvenile specimen MT DF20230601004

weak dorsolateral glandular lines (vs strong dorsolateral folds); differs from *A. tuberodepressus* by having a smaller body size (adult males SVL 43.2–46.8 mm vs 48–56mm), and by having weak dorsolateral glandular lines (vs absent); differs from *A. jinjiangensis* by having distinct tympanum (vs indistinct).

*Amolops dafangensis* sp. nov. is phylogenetically most closed to *A. loloensis* and *A. sangzhiensis*, and the new species could be distinguished from *A. loloensis* by having a smaller body size in males (adult males SVL 43.2–46.8 mm vs 55–62 mm), having distinct tympanum (vs indistinct), tibiotarsal articulation reaching the level far beyond the tip of the snout when leg stretched forward (vs just reaching eye or nostrils), spots on head and dorsum irregular (vs spots on head and dorsum round or oval); differs from *A. sangzhiensis* by having a larger body size in males (adult males SVL 43.2–46.8 mm vs 40.3–40.9 mm), having distinct tympanum (vs indistinct),tibiotarsal articulation reaching the level far beyond the tip of the snout when leg stretched forward (vs just reaching nostrils), mouth corner smooth (vs with dense spiny tubercles around the mouth corner).

*Amolops dafangensis* sp. nov. differs from the species of the *A. monticola* group namely *A. adicola*, *A. akhaorum*, *A. aniqiaoensis*, *A. archotaphus*, *A. bellulus*, *A. binchachaensis*, *chakrataensis*, *A. chaochin*, *A. chunganensis*, *A. compotrix*, *A. cucae*, *A. daorum*, *A. deng*, *A. iri*, *A. kohimaensis*, *A. mengdingensis*, *A. mengyangensis*, *A. monticola*, *A. nyingchiensis*, *A. putaoensis*, *A. truongi*, *A. tuanjieensis*, *A. vitreus*, and *A. wenshanensis* by dorsolateral folds weak formed by series of glands (vs truth dorsolateral folds present), further distinguished from *A. adicola*,

*A. akhaorum*, *A. aniqiaoensis*, *A. archotaphus*, *A. chaochin*, *A. chunganensis*, *A. compotrix*, *A. cucae*, *A. daorum*, *A. iriodes*, *A. kohimaensis*, *A. mengdingensis*, *A. mengyangensis*, *A. monticola*, *A. putaoensis*, *A. truongi*, *A. tuanjieensis*, *A. vitreus*, and *A. wenshanensis* by vocal sac absent (vs present).

*Amolops dafangensis* sp. nov. differs from *A. chayuensis*, the sole member of the *A. chayuensis group*, by dorsolateral folds weak formed by series of glands (vs truth dorsolateral folds present), and vocal sacs absent (vs present).

*Amolops dafangensis* sp. nov. differs from the *A. viridimaculatus* group contains 14 species, namely *A. beibengensis*, *A. chanakya*, *A. formosus*, *A. himalayanus*, *A. kaulbacki*, *A. longimanus*, *A. medogensis*, *A. nidorbellus*, *A. pallasitatus*, *A. senchalensis*, *A. tawang*, *A. wangyali*, *A. wangyufani*, and *A. viridimaculatus* by dorsolateral folds weak formed by series of glands (vs dorsolateral folds absent) and smaller body size (vs male SVL 75.8 mm in *A. beibengensis*, male SVL 76.4 mm in *A. chanakya*, males SVL 61.3–63.1 mm in *A. formosus*, male SVL 80 mm in *A. himalayanus*, males SVL 70–72 mm in *A. kaulbacki*, male SVL 95 mm in *A. medogensis*, males SVL 76.4–82.3 mm in *A. nidorbellus*, male SVL 46.2 mm in *A. senchalensis*, male SVL 82.5 mm in *A. tawang*, males SVL 71.4–76.7 mm in *A. wangyali*, males SVL 68.3–69.0 mm in *A. wangyufani*, and males SVL 72.7–82.3 mm in *A. viridimaculatus*).

*Amolops dafangensis* sp. nov. differs from the *A. marmoratus* group of 13 species (*A. afghanus*, *A. assamensis*, *A. gerbillus*, *A. indoburmanensis*, *A. jaunsari*, *A. latopalmatus*, *A. mahabharatensis*, *A. marmoratus*, *A. nepalicus*, *A. panhai*, *A. siju*, and *A. terraorchis*) by circum-marginal groove on disc of finger I absent (vs present), and vocal sac absent (vs present with the exception of *A. siju*).

*Amolops dafangensis* sp. nov. differs from *A. spinapectoralis*, the sole member of the *A. spinapectoralis* group, by circum-marginal groove on disc of finger I absent (vs present), and vocal sac absent (vs present).

*Amolops dafangensis* sp. nov. differs from the *A. larutensis* group with eight species, namely *A. attiguus*, *A. australis*, *A. cremnobatus*, *A. gerutu*, *A. kottelati*, *A. larutensis*, *A. sengae*, and *A. tanfuilianae* by circum-marginal groove on disc of finger I absent (vs present), and vocal sac absent (vs present).

*Amolops dafangensis* sp. nov. differs from the *A. ricketti* group that contains eight species (*A. shihaitaoi*, *A. sinensis*, *A. ricketti*, *A. wuyiensis*, *A. yunkaiensis*, *A. albispinus*, *A. yatseni*, and *A. tonkinensis*) by circum-marginal groove on disc of finger I absent (vs present), dorsolateral glandular folds present (vs absent), and nuptial pad without conical or papillate nuptial spines (vs present).

*Amolops dafangensis* sp. nov. differs from the *A. daiyunensis* group of three species, namely *A. daiyunensis*, *A. teochewiensis* and *A. teochew*, by circum-marginal groove on disc of finger I absent (vs present), vomerine teeth present (vs absent) and and vocal sac absent (vs present).

*Amolops dafangensis* sp. nov. differs from the *A. hainanensis* group (*A. hainanensis* and *A. torrentis*) by vomerine teeth present (vs absent) and further differs from *A. hainanensis* by having a smaller body size (adult males SVL 43.2–46.8 mm vs 71–93 mm) and circum-marginal groove on disc of finger I absent (vs present); further differs from *A. torrentis* by having a larger body size (adult males SVL 43.2–46.8 mm vs 28–33 mm) and vocal sac absent (vs present).

**Distribution and ecology.** At present, *Amolops dafangensis* sp. nov. was only found on vegetation in a mountain stream in Dafang County, Guizhou Province,

China at approximately 1600 m elevation. The rocks of this stream are covered with moss, and low vegetation grows out of the cracks (Fig. 6). We did not find eggs, nor *Amolops dafangensis* sp. nov. tadpoles or females, and advertisement calls were not recorded, but we observed distinct nuptial pad in the males. Based on our surveys, we speculate that the breeding season is probably in early June. *Boulenophrys jiangi* (Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020), *Boulenophrys qianbeiensis* (Su, Shi, Wu, Li, Yao, Wang & Li, 2020), and *Leptobrachella jinshaensis* Cheng, Shi, Li, Liu, Li & Wang, 2021were also found in the type locality.

**Etymology.** The specific epithet *dafangensis* refers to the distribution of this species, Dafang County, Guizhou Province, China. We propose the common English name "Dafang cascade frogs" for this species and Chinese name as "Da Fang Tuan Wa (大方湍蛙)".

**Figure 6.** Habitat of *Amolops dafangensis* sp. nov. in the type locality, Dafang County, Guizhou Province, China.

## Discussion

In this study, we describe a new species based on morphological comparisons and molecular phylogenetic analyses; although the genetic distance between the new species and its most closely-related congeners is 0.7% for the 16S gene, the morphological characters differ from those of other species of the genus *Amolops*. This small genetic difference is likely due to the limited phylogenetic information content in this particular gene fragment (Chan et al. 2022). Speciation usually begins with spatial isolation or adaptation to unique environments without strict isolation (Schilthuizen 2000). Significant spatial isolation and subsequent formation of unique lineages may be due to isolation or long-range dispersal across barriers such as mountains, rivers, or other intervening unsuitable habitats (Mayr 1963; Avise 2000; Rundle and Nosil 2005; Schluter 2009). The geographical distances between *Amolops dafangensis* sp. nov. and

its closely-related congeners *A. sangzhiensis* and *A. loloensis* are more than 800 km and 370 km, respectively, and the type locality of the three species are in different mountains: the new species is distributed in Dalou Mountains, *A. sangzhiensis* in easternmost Wuling Mountains, and *A. loloensis* in the Daliangshan Mountains, with significantly different biota. Therefore, we speculate that isolation is likely to have promoted speciation between the lineages and led to the evolution of different morphologies between the new species, *A. sangzhiensis*, and *A. loloensis*.

In the last five years, 25 new frog species have been described in Guizhou Province, China (Frost 2023). Dafang County is in the northwest of Guizhou Province, China, and there have been few surveys of amphibians in the area over the years. From 2020 to 2023 we conducted five surveys in this region. Only in June 2023 was the new species discovered, and only three adult males and two juveniles were found in a range of ~ 100 meters below the source of the stream. Therefore, we infer that the population of the new species is small. We recommend the new species be assigned as vulnerable (VU) according to the evaluation criteria of the IUCN Red List of threatened Species (IUCN 2012). Future research should focus on determining the distribution and elevational range of the species.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This work was supported by the Projects from the West Light Foundation of The Chinese Academy of Sciences (Grant No. 2021XBZG_XBQNXZ_A_006), the National Natural Science Foundation of China (Nos. 32270498, 31960099, 32260136, and 32070426), Guizhou Provincial Science and Technology Projects (Nos. ZK[2022]540 and [2023] 099), Forestry Science and Technology Research Project of Guizhou Forestry Department (No. [2020]13, [2020]04); Guizhou Provincial Department of Education Youth Science and Technology Talents Growth Project (Nos. KY[2020]234 and KY[2020]237), and High-level personnel research start-up funding projects of Moutai Institute (Nos. mygccrc[2022]055, mygccrc[2022]067, mygccrc[2022]083).

### Author contributions

Funding acquisition: GC. Investigation: JL, XCK. Writing - original draft: SZL. Writing - review and editing: BW.

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### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Uncorrected p-distances between species in the *Amolops mantzorum* group based on the 16S gene sequences

Authors: Shi-Ze Li, Jing Liu, Xiao-Cong Ke, Gang Cheng, Bin Wang

Data type: xlsx

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Link: https://doi.org/10.3897/zookeys.1189.115621.suppl1

## Supplementary material 2

### Uncorrected p-distances between species in the *Amolops mantzorum* group based on the COI gene sequences

Authors: Shi-Ze Li, Jing Liu, Xiao-Cong Ke, Gang Cheng, Bin Wang

Data type: xlsx

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